Supporting Information

Neuroprotective Activities of Heparin, Heparinase III, and Hyaluronic Acid on the Aβ42-treated Forebrain Spheroids Derived from Human Stem Cells

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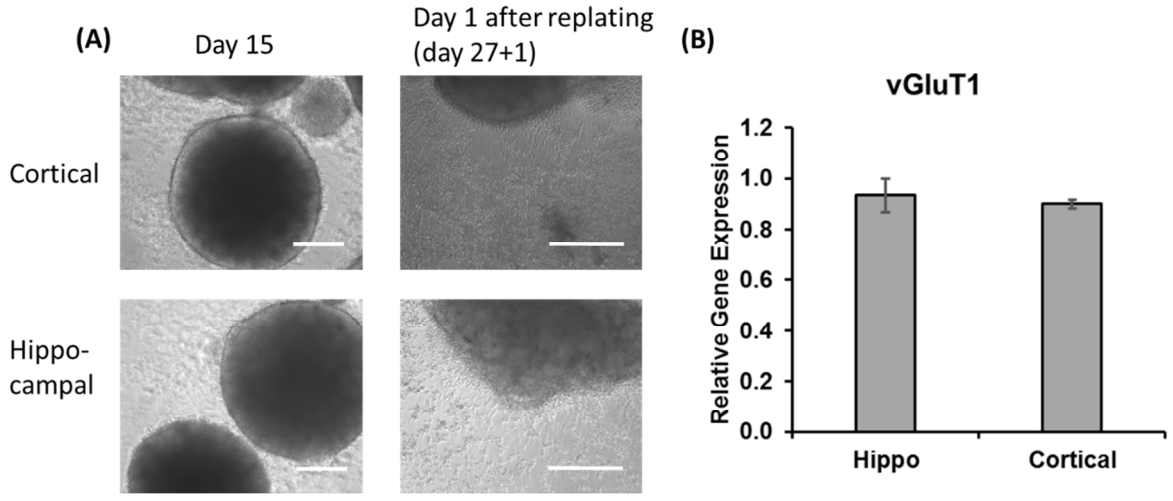
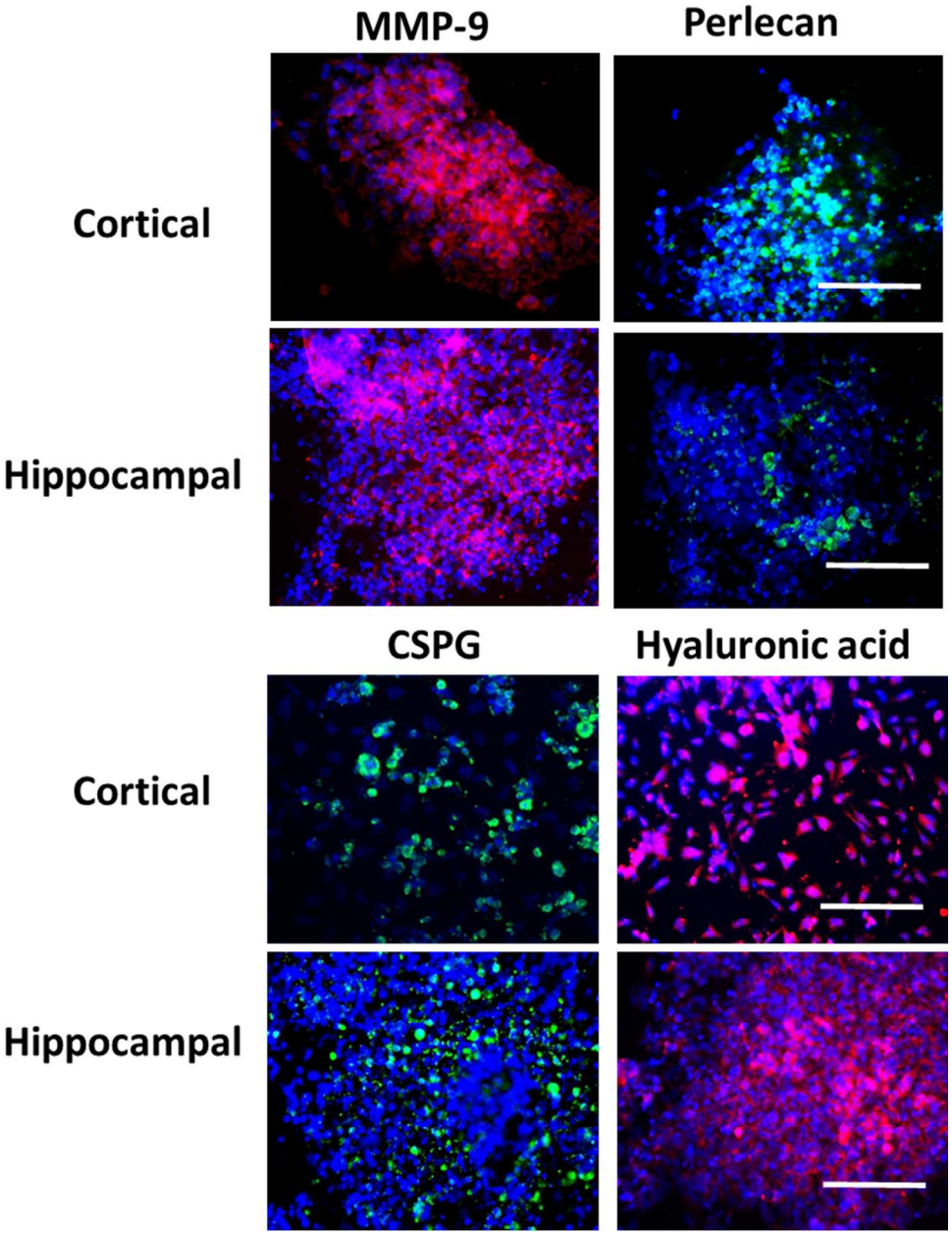
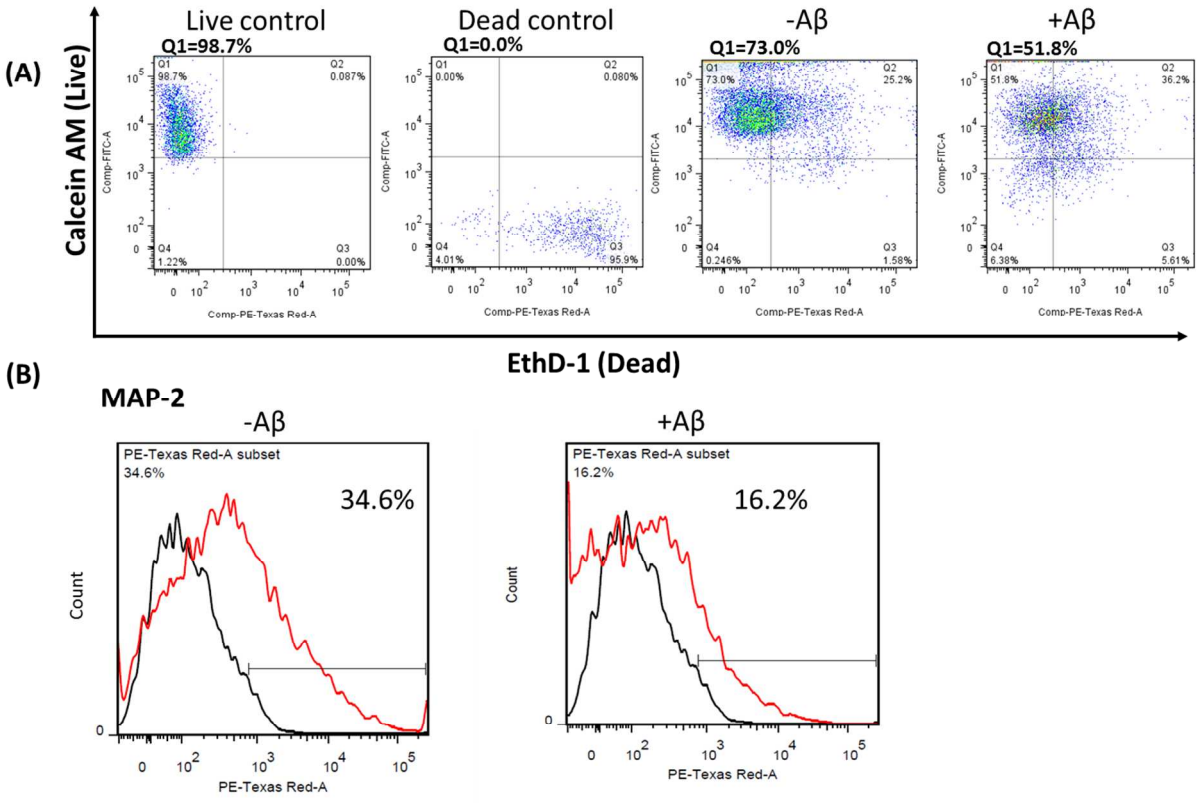
Supplementary Figure S1. **Characterization of cortical and hippocampal differentiation from human induced pluripotent stem (iPS) cells.** (A) Phase contrast images of cortical spheroids and hippocampal spheroids and the replated spheroids. Scale bar: 200 μm. (B) RT-PCR analysis of vGlut1 gene expression. Neural spheroids derived with cortical and hippocampal protocols were compared.



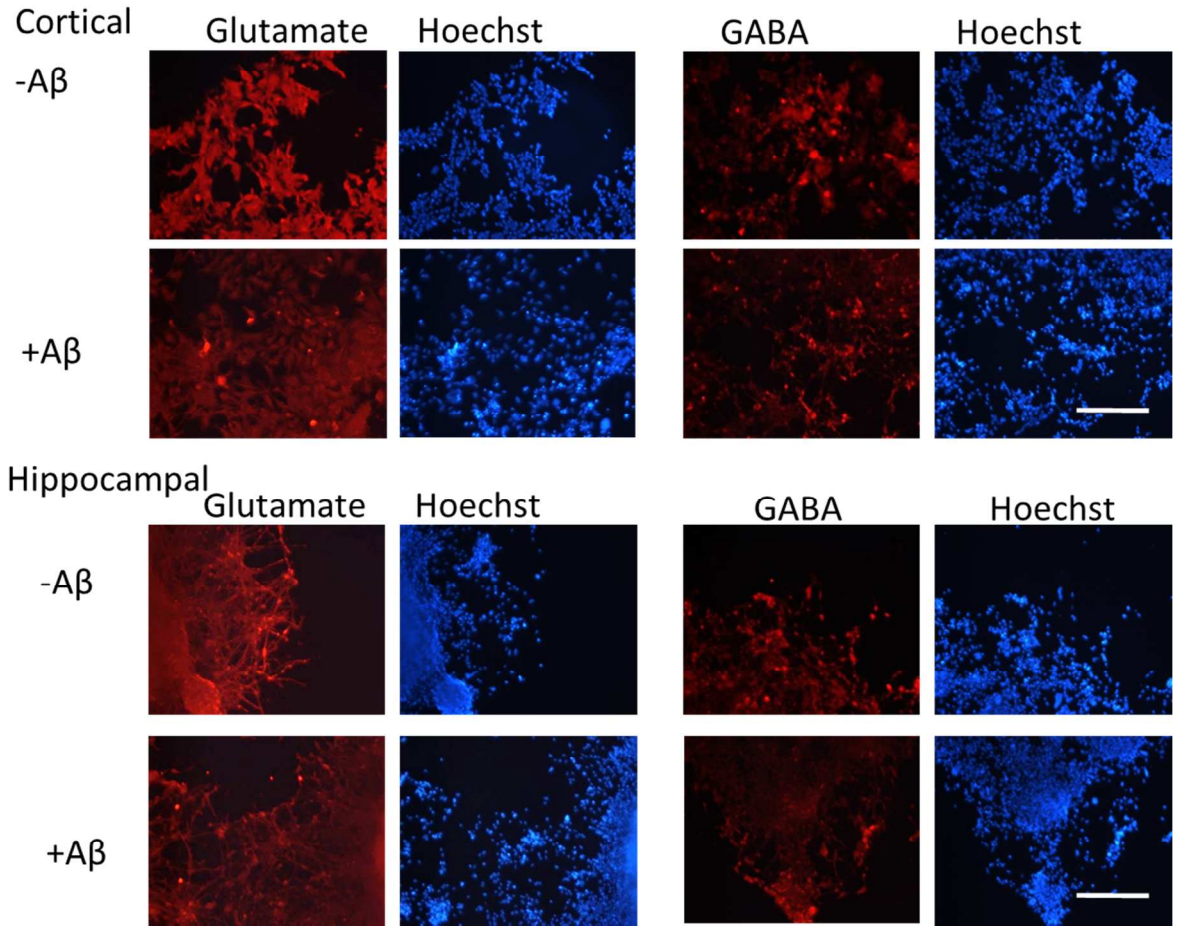
Supplementary Figure S2. **Expression of MMP-9, Perlecan (a HSPG protein), CSPG, and hyaluronic acid (HA) for cortical and hippocampal groups.** Scale bar: 100 μm. The expression pattern showed circular signals around the cells or deposition among the cells.



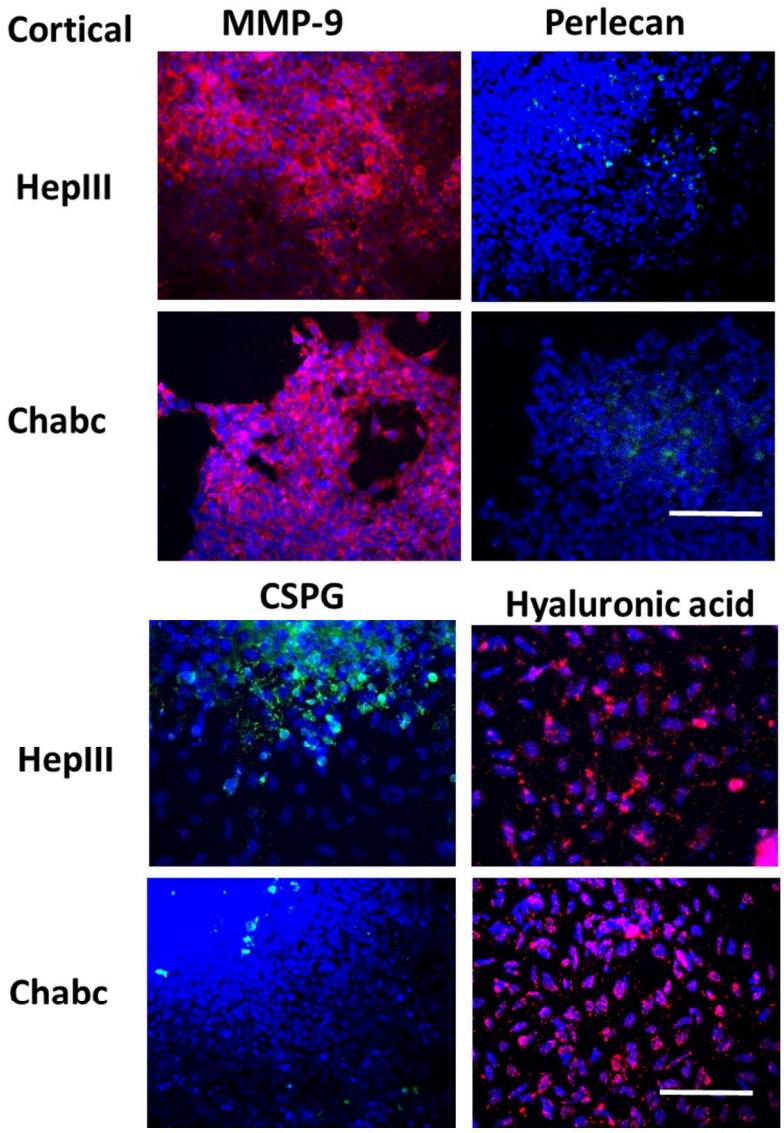
Supplementary Figure S3. **Effect of Aβ42 treatment.** **(**A) Two-color flow cytometry analysis of the Live/Dead cells showing decreased percentage of viable cells after Aβ42 treatment. (B) Flow cytometry analysis of MAP2 showing decreased neuron population after Aβ42 treatment. Black line: negative control. Red line: marker of interest.



Supplementary Figure S4. **Glutamate and GABA expression for cortical and hippocampal cells after Aβ42 treatment.** Red: Glutamate or GABA. Blue: Hoechst. Scale bar: 100 μm.



Supplementary Figure S5. Effects of Chabc and heparinase III on expression of MMP-9, Perlecan, CSPG, and HA. The cortical population was treated with heparinase III (HepIII) and Chabc (non-treated group can be seen Supplementary Figure S2). Scale bar: 100 μm. MMP-9 expression was not affected by the enzymatic treatment. HepIII treatment reduced the expression of Perlecan and HA. Chabc treatment reduced the expression of Perlecan and CSPG, and altered HA expression pattern (more dot-like).



Supplementary Table S1. A list of antibodies.

Cells	Primary Antibody	Origin/ Isotype	Supplier/ Cat#	Dilution
Cortical layers	TBR1 (layer VI)	Rabbit IgG	ABCAM, ab31940	1:200
	BRN2 (layer III)	Goat IgG	Santa Cruz, sc-6029	1:200
Dentate gyrus	PROX1	Rabbit IgG	ABCAM, ab101851	1:500
Neurons	β-tubulin III	Mouse IgG_1	Millipore, MAB1637	1:200
	MAP-2	Rabbit IgG	ABCAM, ab32454	1:200
	Glutamate	Rabbit IgG	Sigma, G6642	1:1000
	GABA	Rabbit IgG	Sigma, A2052	1:1000
Pathway	Aβ42	Rabbit IgG	ABCAM, ab10148	1:200
	Tau	Mouse IgG_1	Sigma, T9450	1:100
	Phospho-PHF-tau pSer202+Thr205 (AT8)	Mouse IgG_1	Thermal Fisher, MN1020	1:200
ECM-related	MMP-9	Goat IgG	Santa Cruz, sc-6840	1:200
	Perlecan	Mouse IgG_1	Santa Cruz, sc-377219	1:50
	CSPG	Mouse IgM	Life Technologies, MA1-83055	1:100
	Hyaluronic acid	Sheep IgG	Life Technologies, PA1-85561	1:50
Secondary	Alexa 488, goat anti-mouse IgG_1	-	Life Technologies, A-21121	1:200
	Alexa 488, goat anti-rabbit IgG	-	Life Technologies, A-11008	1:200
	Alexa 488, goat anti-mouse IgM	-	Life Technologies, A-21042	1:200
	Alexa 594, goat anti-rabbit IgG	-	Life Technologies, A-11012	1:400
	Alexa 594, donkey anti-goat IgG	-	Life Technologies, A-11058	1:400
	Alexa 594, donkey anti-sheep IgG	-	Life Technologies, A-11016	1:400

Supplementary Table S2. Primer sequence for target genes.

Gene	Forward primer 5' to 3'	Reverse primer 5' to 3'
TBR1	CCCCCTCGTCTTTCTCTTACC	TAATGTGGAGGCCGAGACTTG
vGluT1	CCCCAATTCCTCGCACTTTAT	GGGAAGGATCCCAGATTTTGA
PROX1	GACTTTGAGGTTCCAGAGAGA	TGTAGGCAGTTCGGGGATTTG
Beta-actin	GTACTCCGTGTGGATCGGCG	AAGCATTTGCGGTGGACGATGG